Commentary

Environmental Health Perspectives
Vol. 48, pp. 137-139, 1983

Protocol for Testing the Toxicity of Chemical Mixtures

by Robert A. Neal*

The purpose of this paper is to suggest those toxicological procedures which may be performed to evaluate whether a significant health risk is posed by exposure of man to a mixture of chemicals that may be present in a particular environment because of improper chemical waste disposal practices. The emphasis of the discussion will be on unusual circumstances of excessive exposure of humans to mixtures of chemicals. The protocol proposed is intended to represent a pragmatic approach to the problem of assessing the potential toxicity to man of exposure to mixtures of chemicals. It should be evident that with current techniques and the present state of knowledge there is no ideal way of approaching the problem of assessing the toxicity of exposure of man to mixtures of chemicals. It should also be evident that absolute answers relative to the potential risk to man from exposure to individual chemicals, much less mixtures of chemicals, is a goal which will not be obtainable in the foreseeable future.

Although the current interest and the majority of the resources of regulatory agencies within the United States are devoted to examining for the presence of carcinogenic and mutagenic chemicals in the environment, it is essential to recognize that chemicals or mixtures of chemicals may also produce acute effects, adverse effects on various organ systems including the hematopoietic and nervous system, effects on the immune system, teratogenic effects and effects on reproduction. Consequently, any protocol for evaluation of the potential adverse health effects of exposure to mixtures of chemicals in a particular environment must take into account all of the potential adverse health effects which may be posed by the organic and inorganic chemicals, particulates and radiochemicals present in the particular environment.

*Chemical Industry Institute of Toxicology, P. O. Box 12137, Research Triangle Park, NC 27709.

The methodology currently available for monitoring for toxic effects or potential toxic effects of exposure of man to mixtures of chemicals include monitoring for individual toxic chemicals in air, drinking water and soil; animal studies with elevated doses of compounds or mixtures of compounds detected by monitoring; "short-term" tests and epidemiological studies of the exposed populations.

Monitoring for individual chemicals is an important activity relative to the assessment of the potential toxicity to man of exposure to a mixture of chemicals as a result of improper waste disposal practices. The identification of known toxic compounds and the level of these compounds to which man may be exposed is an important part of the process of determining whether an adverse health risk is posed by the levels of those chemicals present in the environment. Short-term tests are valuable for assessing the presence of potentially mutagenic or carcinogenic chemicals in the environment in question. Unfortunately, these tests are only available for detecting potential mutagens and carcinogens. In addition, the short-term tests do not usually provide valid quantitative information for use in determining the degree of risk to man from exposure to individual chemicals and mixtures of chemicals which may be potential human mutagens or carcinogens. Although epidemiology offers the most relevant information concerning the potential health risk to man from exposure to a chemical or a mixture of chemicals it suffers from a lack of sensitivity primarily because the size of the population under investigation is almost invariably small. All things considered, the most sensitive and reliable procedures available for assessing the potential toxicity to man from exposure to a mixture of chemicals in a particular environment are studies in experimental animals. The available data suggest that the use of experimental animals in the screening of compounds and mixtures of compounds for

potential toxicity to man has served us well in preventing exposure to chemicals and chemical mixtures with a wide variety of toxic effects. In my opinion, the use of experimental animals in examining for the potential toxicity of exposure of man to a mixture of chemicals as a result of improper chemical waste disposal practices will provide information not as readily available as a result of monitoring for known toxic chemicals, use of short-term tests or epidemiological studies.

As noted previously, the chemicals to which humans may be exposed may be classified into four general types: organic chemicals, inorganic chemicals, particulates and radiochemicals. The particulate which is of greatest health concern is asbestos. It is clear that inhalation of asbestos leads to adverse health effects in man including lung disease and induction of pulmonary cancer. However, it is not clear at this time whether ingested asbestos poses a significant adverse health risk. Of those inorganic anions and cations to which man may potentially be exposed, those of greatest health concern are nickel, arsenic, cadmium, chromium, lead and mercury. Man may occasionally be exposed to other inorganic anions and cations of some health concern. However those listed above, because of their toxic properties, pose the most significant adverse health risk to man. The potential adverse health risks posed by exposure of man to asbestos, inorganic chemicals and radiochemicals as a result of improper disposal can best be controlled by monitoring for the levels of these materials in air, finished drinking water and land surface areas. Using these monitoring data, an estimate of the level of exposure of man to these specific compounds can be obtained. Once the exposure levels have been determined, they can be compared with MCLs, TLVs or radioemission limits which have been set for these compounds or types of radiation detected. On the basis of this comparison, the proper authorities can take the necessary action to terminate or prevent exposure to levels of these compounds which are toxic or potentially toxic.

If significant exposure to two or more toxic inorganic chemicals is detected in the particular environment, an assessment of the potential toxicity of the combined exposure should be made. If no data are available concerning the toxic effects of the combined exposure, consideration should be given to carrying out animal studies with the mixture of inorganic chemicals to determine if the toxic effects of each are synergized, additive or antagonized.

In comparison to inorganic chemicals, particulates and radiochemicals, the evaluation of the potential adverse health effect posed by exposure to mixtures of organic chemicals in a particular environment is a much more complex task. Monitoring surveys carried out by the Environmental Protection Agency and others have detected in excess of a thousand different organic chemicals in various finished drinking water supplies in the United States. In addition, monitoring studies carried out in the vicinity of certain chemical disposal sites have identified the existence of human exposure to mixtures of organic chemicals. Some of the chemicals detected in these monitoring activities have known toxicity. In the case of drinking water, with few exceptions, these compounds with known or suspected toxicity are either detected infrequently or are present in very low concentrations. It is an unusual event when adverse human health effects occur on exposure to trace amounts of chemicals. In the vast majority of the recorded cases, human toxicity has resulted from excessive exposure to the chemical or mixture of chemicals in question.

Therefore, this discussion will focus on the determination of the potential toxicity resulting from exposures to relatively high concentrations of chemicals. To do otherwise is impractical. To identify each organic compound present in detectable concentrations in a particular environment and examine its toxicity through a complete battery of animal and "short-term" tests is also not practical.

The recommended strategy for detecting the toxicity or potential toxicity of exposure to a mixture of organic chemicals in a particular environment is to first monitor for organic chemicals of known or suspected toxicity to man and compare the levels of exposure to these compounds to MCLs, TLVs and other estimates of exposure level without significant toxicity (real or estimated). Of course the choice of what chemicals to examine for is greatly facilitated by knowing something about what chemicals were disposed of in that particular environment. On the basis of the monitoring data and a comparison of the level of compounds of known or suspected toxicity to man with MCLs and TLVs, the proper authorities can take what action is deemed necessary to terminate or prevent exposure to levels of these compounds which are toxic or potentially toxic to humans.

Epidemiology is an important aspect of monitoring the exposed human populations for potential toxic effects from exposures to mixtures of organic chemicals in a particular environment. If in a particular incidence, there is a population which has been exposed or will continue to be exposed to compounds of known or suspected toxicity then it would be important to maintain, if possible, a registry of mortality, cancer incidence and type, data on reproductive efficiency, congenital malfor-

mations, school absentee records and hospital discharge records of the exposed populations.

Invariably, in case of human exposure to mixtures of organic chemicals, the toxicologist will have to evaluate the potential toxic effects to man of exposure to organic compounds which have not been adequately evaluated using epidemiological investigations, animal studies or short-term tests. In addition, uncertainty may exist concerning whether simultaneous exposure to multiple compounds may result in the synergism of the toxicity of compounds of known or suspected toxicity. These considerations may lead to concern about the levels of exposure of man to compounds of unknown toxicity in the mixture of organic chemicals and uncertainty about the results of the epidemiological surveys. If there is doubt about the toxic effects which may have occurred because of exposure to the mixture of organic chemicals and concern about the advisability of continued human exposure to the mixture, consideration should be given to exposing experimental animals to elevated levels of a "representative" mixture of the chemicals to which humans may be exposed examining for acute, subacute (organ system toxicity, teratogenicity, reproductive toxicity), and chronic (organ system toxicity, carcinogenicity) toxicity. In these studies the experimental animals should be exposed to the mixture of compounds by the same route by which humans are exposed. In other words, if humans are primarily exposed by inhalation, the exposure of the animals to the mixture ought to be by inhalation as well. The "representative" mixture should also be examined for genetic toxicity (mutagenic activity, clastogenic activity, unscheduled DNA synthesis, etc.). The major question which arises is what is a "representative" mixture of chemicals to which humans may be exposed? Does one include all of the chemicals which have been detected in the monitoring? In my opinion, only those chemicals which have been detected to be present in significant amounts (parts per billion or greater) should be included in the "representative" mixture. An exception would be if exposure has occurred to trace amounts of highly toxic chemicals such as the chlorinated dioxins or dibenzofurans, consideration should be given to including these chemicals in the mixture. If positive results are obtained in these animal studies, then the proper authorities should take whatever action is deemed necessary to terminate or prevent exposure to levels of the mixture of chemicals which are potentially toxic to humans.

In examining the proposed protocol, it is clear that there are limitations to the strategies outlined. For example, in the case of the proposed epidemiological studies, confounding factors such as smoking, diet, occupation, etc. will make interpretation of the results of the epidemiological studies difficult. In addition, one would expect difficulty in identifying known toxic compounds in a complex mixture of organic compounds. For example, a tetrachlorodibenzo-*p*-dioxin may be detected but it may be difficult to determine whether the tetrachlorodibenzo-*p*-dioxin detected is a toxic isomer such as 2,3,7,8 or another isomer without known toxicity, for example, 1,3,6,8. The major limitation, however, is deciding what is the "representative" mixture of organic chemicals to be used in the animal studies. Deciding which compounds, present in small amounts in the mixture of organic compounds, should not be included in the "representative" mixture will be a difficult task. The expense of the animal studies must also be considered a limitation as well. Finally, the extrapolation of the results of the animal studies to potential results in man is a limitation of the proposed protocol. This is a limitation that is common to all animal testing for the toxicity of chemicals. However, in spite of these limitations, animal testing contains more of the elements of sensitivity and predictability than any other methodology currently available to assess the toxicity or potential toxicity to man from exposure to mixtures of chemicals present in low levels in a particular environment.